

# Draft Genome Sequences of *Ralstonia pickettii* Strains SSH4 and CW2, Isolated from Space Equipment

Pieter Monsieurs,<sup>a</sup> Kristel Mijndonckx,<sup>a</sup> Ann Provoost,<sup>a</sup> Kasthuri Venkateswaran,<sup>b</sup> C. Mark Ott,<sup>c</sup> Natalie Leys,<sup>a</sup> Rob Van Houdt<sup>a</sup>

Unit of Microbiology, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium<sup>a</sup>; Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA<sup>b</sup>; Biomedical Research and Environmental Science Division, NASA, Johnson Space Center, Houston, Texas, USA<sup>c</sup>

***Ralstonia pickettii* SSH4 and CW2 were isolated from space equipment. Here, we report their draft genome sequences with the aim of gaining insight into their potential to adapt to these environments.**

Received 6 August 2014 Accepted 8 August 2014 Published 4 September 2014

**Citation** Monsieurs P, Mijndonckx K, Provoost A, Venkateswaran K, Ott C, Leys N, Van Houdt R. 2014. Draft genome sequences of *Ralstonia pickettii* strains SSH4 and CW2, isolated from space equipment. *Genome Announc.* 2(5):e00887-14. doi:10.1128/genomeA.00887-14.

**Copyright** © 2014 Monsieurs et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Rob Van Houdt, rob.van.houdt@sckcen.be.

*Ralstonia pickettii* SSH4 and CW2 are Gram-negative bacteria (*Burkholderiaceae* family) isolated pre-flight from the surface of the Mars Odyssey Orbiter during assembly at the Kennedy Space Center in Florida and from a water sample taken in-flight from the American segment of the International Space Station (ISS) cooling system, respectively (1–3).

*R. pickettii* strains are prevalent in water and soil (4). They have been recovered from many different water sources such as distilled water used in hospitals (5), dental unit water lines (6), public drinking water supplies (7), bottled water (8), ultrapure industrial water systems (9, 10), and even from drinking water systems of the Mir space station (11) and the Shuttle (12). *R. pickettii* has the ability to survive and thrive in oligotrophic conditions (4, 13) probably mediated by its biodegradative abilities (4), its large metabolic diversity, and its ability to form biofilms, making them more resistant to biocides and consequently more difficult to eradicate (14–16). In addition, *R. pickettii* has been recovered from a wide range of clinical environments and emerged as an opportunistic pathogen that should not be overlooked as a cause of nosocomial infections (17). The draft genome sequences reported here will help to elucidate how these strains are able to persist in these strictly controlled environments.

Whole-genome shotgun and paired-end sequencing of *R. pickettii* SSH4 and CW2 were performed by Macrogen (Seoul, Korea) using the 454 GS FLX sequencing platform. The sequencing data showed an average read length of 331 nt and 332 nt, an average insert size of 2877 nt and 2826 nt, and a total number of sequencing data of 367 Mbp and 337 Mbp for strains SSH4 and CW2, respectively. Both genomes were assembled using the Newbler software (version 2.3) resulting in 38 and 32 contigs and  $N_{50}$  values of 534,775 nt and 469,201 nt for SSH4 and CW2, respectively.

The genome of *R. pickettii* SSH4 was estimated to be 5,746,538 bp with a G+C content of 63.30%. The genome of *R. pickettii* CW2 was estimated to be 5,490,874 bp with a G+C content of 63.65%. Both strains harbor a chromosome, a chromid (18), and a megaplasmid (>230 kb). Strain SSH4 carries two additional plasmids of around 65 and 95 kb, respectively. These estimations were based on plasmid extraction and gel electrophore-

sis analysis as previously reported (1). The automated genome annotation of SSH4 through the MicroScope platform (19) identified 5,868 protein-coding genes of which 72.0% were classified in at least one cluster of orthologous group (COG), 49 tRNA genes, and 3 rRNA genes. The CW2 genome annotation displayed 5,598 protein-coding genes of which 73.7% were classified in at least one COG, 51 tRNA genes, and 3 rRNA genes. A general pan-genome analysis via the MicroScope platform (19) indicated that SSH4 and CW2 share 3,903 coding sequences.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers [JFZG000000000](https://www.ncbi.nlm.nih.gov/nuccore/JFZG000000000) and [JFZH000000000](https://www.ncbi.nlm.nih.gov/nuccore/JFZH000000000) for strains SSH4 and CW2, respectively.

## ACKNOWLEDGMENTS

This work was supported by the European Space Agency (ESA-PRODEX) and the Belgian Science Policy (Belspo) through the COMICS project (C90356).

## REFERENCES

- Mijndonckx K, Provoost A, Ott CM, Venkateswaran K, Mahillon J, Leys N, Van Houdt R. 2013. Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. *Microb. Ecol.* 65:347–360. <http://dx.doi.org/10.1007/s00248-012-0139-2>.
- La Duc MT, Nicholson W, Kern R, Venkateswaran K. 2003. Microbial characterization of the Mars Odyssey spacecraft and its encapsulation facility. *Environ. Microbiol.* 5:977–985. <http://dx.doi.org/10.1046/j.1462-2920.2003.00496.x>.
- Benardini J, Ballinger J, Crawford R, Roman M, Sumner R, Venkateswaran A. 2005. International Space Station internal active thermal control system: an initial assessment of the microbial communities within fluid from ground support and flight hardware. SAE Technical Paper 2005-01-3094. <http://dx.doi.org/10.4271/2005-01-3094>.
- Ryan MP, Pembroke JT, Adley CC. 2007. *Ralstonia pickettii* in environmental biotechnology: potential and applications. *J. Appl. Microbiol.* 103: 754–764. <http://dx.doi.org/10.1111/j.1365-2672.2007.03361.x>.
- Kendirli T, Ciftçi E, Ince E, Incesoy S, Güriz H, Aysev AD, Tutar E, Yavuz G, Dogru U. 2004. *Ralstonia pickettii* outbreak associated with contaminated distilled water used for respiratory care in a paediatric intensive care unit. *J. Hosp. Infect.* 56:77–78. <http://dx.doi.org/10.1016/j.jhin.2003.09.011>.

6. Szymańska J. 2006. Bacterial decontamination of DUWL biofilm using Oxygenal 6. *Ann. Agric. Environ. Med.* 13:163–167.
7. Lee J, Lee CS, Hugunin KM, Maute CJ, Dysko RC. 2010. Bacteria from drinking water supply and their fate in gastrointestinal tracts of germ-free mice: a phylogenetic comparison study. *Water Res.* 44:5050–5058. <http://dx.doi.org/10.1016/j.watres.2010.07.027>.
8. Falcone-Dias MF, Vaz-Moreira I, Manaia CM. 2012. Bottled mineral water as a potential source of antibiotic resistant bacteria. *Water Res.* 46:3612–3622. <http://dx.doi.org/10.1016/j.watres.2012.04.007>.
9. Bohus V, Tóth EM, Székely AJ, Makk J, Baranyi K, Patek G, Schunk J, Márialigeti K. 2010. Microbiological investigation of an industrial ultra pure supply water plant using cultivation-based and cultivation-independent methods. *Water Res.* 44:6124–6132. <http://dx.doi.org/10.1016/j.watres.2010.07.006>.
10. Kulakov LA, McAlister MB, Ogden KL, Larkin MJ, O'Hanlon JF. 2002. Analysis of bacteria contaminating ultrapure water in industrial systems. *Appl. Environ. Microbiol.* 68:1548–1555. <http://dx.doi.org/10.1128/AEM.68.4.1548-1555.2002>.
11. Baker PW, Leff L. 2004. The effect of simulated microgravity on bacteria from the Mir space station. *Microgravity Sci. Technol.* 15:35–41. <http://dx.doi.org/10.1007/BF02870950>.
12. Koenig DW, Pierson DL. 1997. Microbiology of the space shuttle water system. *Water Sci. Technol.* 35:59–64. [http://dx.doi.org/10.1016/S0273-1223\(97\)00235-7](http://dx.doi.org/10.1016/S0273-1223(97)00235-7).
13. McAlister MB, Kulakov LA, O'Hanlon JF, Larkin MJ, Ogden KL. 2002. Survival and nutritional requirements of three bacteria isolated from ultrapure water. *J. Ind. Microbiol. Biotechnol.* 29:75–82. <http://dx.doi.org/10.1038/sj.jim.7000273>.
14. Dombrowsky M, Kirschner A, Sommer R. 2013. PVC-piping promotes growth of *Ralstonia pickettii* in dialysis water treatment facilities. *Water Sci. Technol.* 68:929–933. <http://dx.doi.org/10.2166/wst.2013.332>.
15. Anderson RL, Holland BW, Carr JK, Bond WW, Favero MS. 1990. Effect of disinfectants on pseudomonads colonized on the interior surface of PVC pipes. *Am. J. Public Health* 80:17–21. <http://dx.doi.org/10.2105/AJPH.80.1.17>.
16. Van Houdt R, Michiels CW. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *J. Appl. Microbiol.* 109: 1117–1131. <http://dx.doi.org/10.1111/j.1365-2672.2010.04756.x>.
17. Ryan MP, Pembroke JT, Adley CC. 2006. *Ralstonia pickettii*: a persistent gram-negative nosocomial infectious organism. *J. Hosp. Infect.* 62: 278–284. <http://dx.doi.org/10.1016/j.jhin.2005.08.015>.
18. Van Houdt R, Mergeay M. 2012. Plasmids as secondary chromosomes. In Bell E, Bond J, Klinman J, Masters B, Wells R (ed), *Molecular life sciences: an encyclopedic reference*. Springer-Verlag, Berlin, Germany.
19. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Fèvre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C. 2013. Microscope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res.* 41:D636–D647. <http://dx.doi.org/10.1093/nar/gks1194>.